Novel protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines

C L A I M S

1. Protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines having the following properties wherein said protein has to comply with at least the features a), b), c), and d):

a) may be isolated from murine myelomonocytic leukemia cell lines;

b) may be isolated from irradiated human bone marrow stromal cell lines;

c) induces differentiation in Friend erythroleukemia cell lines with hemoglobin formation;

d) having a molecular weight in the range of about 10 - 60 kDa as determined by gel filtration on Sephacryl S300®;

e) with an expression of the corresponding mRNA in primary cells of the thymus, fetal liver, adult spleen, or bone marrow;

f) with characteristic repeat structures in the cDNA encoding the protein;

g) with corresponding mRNA species of different length consisting of identical 3´ regions but different 5´ regions.

2. Protein according to claim 1, characterized in that said protein has at least one of the following features:

h) showing a stable in vitro expression of the corresponding mRNA if an allogenic spleen cell reaction is carried out with non-irradiated, not pretreated spleen cells of mouse strains CBA and C57Bl/6;

i) having AT rich regions in the cDNA encoding the protein;

k) inducible by a serum factor present in fetal calf serum.

3. Protein according to claim 1, characterized in that

one or more of the repeat sequences presented in Table 3 or of repeat sequences hybridizing to these repeat sequences under stringent conditions are present in the DNA encoding the protein of claim 1 or claim 2.

4. Protein according to one or more of the preceding claims,
characterized in that
said protein may be isolated from human cells, murine cells, or the culture supernatants of human or murine cell lines.

5. Protein according to one or more of the preceding claims,
characterized in that
said protein exhibits a partial amino acid sequence encoded by a DNA hybridizing to the cDNA of SEQ ID NO:1 or NO:2 or NO:4.

6. Protein according to one or more of the preceding claims,
characterized in that
said protein exhibits a partial amino acid sequence encoded by a DNA hybridizing to the cDNA of SEQ ID NO:1 or NO:2 or NO:4 under stringent conditions.

7. Protein according to one or more of the preceding claims,
characterized in that
there are also comprised portions, analogues, and derivatives of said protein as well as fusion proteins each coding for a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines.

8. Protein according to one or more of the preceding claims having an essentially purified, native form.

9. Protein according to one or more of the preceding claims having an essentially recombinant form.

10. Protein according to one or more of the preceding claims said protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines and/or growth factor activity and/or colony-stimulating activity.

11. Protein according to one or more of the preceding claims,
characterized in that
said protein exhibits a differentiation-inducing effect on human leukemia cell lines.

12. Protein according to one or more of the preceding claims,
characterized in that
said protein contains partial amino acid sequences according to SEQ ID NO:3 or NO:5 wherein one or more of the amino acids may be deleted, substituted, or added each having at least differentiation-inducing activity on Friend erythroleukemia cell lines.

13. DNA fragment according to SEQ ID NO:1 or NO:2 or NO:4 or the complementary strand thereof, portions, derivatives, and analogues thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines.

14. DNA fragments, portions, analogues, and derivatives thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines hybridizing to the cDNA according to SEQ ID NO:1 or NO:2 or NO:4 and/or which are degenerated by the genetic code.

15. DNA fragments, portions, analogues, and derivatives

thereof each coding for a polypeptide having at least differentiation-inducing activity on Friend erythroleukemia cell lines hybridizing to the cDNA according to SEQ ID NO:1 or NO:2 or NO:4 under stringent conditions and/or which are degenerated by the genetic code.

16. DNA fragment of one or more of the preceding claims, characterized in that
said DNA fragment encodes at least a part of a polypeptide with the activity of the human or murine protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to one or more of the preceding claims.

17. Recombinant vector,
characterized in that
said vector contains a DNA sequence corresponding to a gene or a DNA fragment encoding the protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines according to ~~one or more of the preceding~~ claims.

18. Recombinant vector according to one or more of the preceding claims,
characterized in that
said vector is derived from a bacterial plasmid, a bacteriophage, or a viral vector.

19. Host cell transformed by a vector according to ~~one or more of the preceding~~ claims.

20. Host cell according to claim 19,
characterized in that
said host cell is a prokaryotic cell or an eukaryotic cell.

21. Method for the preparation of a DNA fragment according

to one or more of the preceding claims, characterized in that said fragment comprises screening of a human or murine cDNA clone library using as a probe a DNA fragment of a DNA coding for a murine or human protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines.

22. Monoclonal or polyclonal antibody directed against at least one epitope of a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to one or more of the preceding claims.

23. Therapeutic, diagnostic or experimentally useful means, characterized in that said means contains as an effective substance at least one nucleic acid in an effective amount which hybridizes to a gene or a part thereof encoding the protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines according to ~~one or more of the preceding~~ claim 1.

24. Means according to one or more of the preceding claims, characterized in that said means contains as an effective substance at least one nucleic acid comprising (a) the nucleotide sequence encoding a protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines, (b) a portion thereof, (c) a nucleotide sequence hybridizing to a nucleic acid as under (a) and/or (b) under stringent conditions, or (d) a nucleotide sequence complementary to a nucleotide sequence as under (a), (b), and/or (c).

25. Means according to one or more of the preceding claims, characterized in that said nucleic acid optionally is a modified DNA.

26. Means according to one or more of the preceding claims, characterized in that
said nucleic acid optionally is a modified RNA.

27. Therapeutic means,
characterized in that
said means contains a protein, an analogue, a derivative or portions thereof according to ~~one or more of the preceding~~ claims 1, each functioning as a polypeptide with at least differentiation-inducing activity on Friend erythroleukemia cell lines together with conventional carriers and excipients in an effective amount.

28. Use of a means according to one or more of the preceding claims as a molecular probe in diagnostics or therapy.

29. Use of a means according one or more of the preceding claims as an antisense nucleic acid for the inhibition of gene expression.

30. Use of a DNA encoding a protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines, a portion, derivative, or analogue thereof each functioning as a polypeptide with at least differentiation-inducing activity on Friend erythroleukemia cell lines for the incorporation into a prokaryotic or eukaryotic cell.

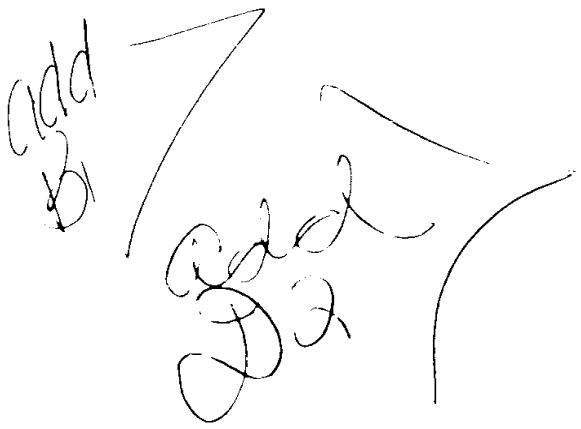
31. Fusion protein having an amino acid sequence consisting completely or in part of the amino acid sequence of the human or murine protein with at least differentiation-inducing activity on Friend erythroleukemia cell lines according to one or more of the preceding claims and in part of a prokaryotic and/or eukaryotic protein.

32. Synthetic protein having at least differentiation-inducing activity on Friend erythroleukemia cell lines

33. Use of a protein according to one or more of the preceding claims or of inhibitors of said protein in the treatment of diseases in which a local or systemic overproduction or underproduction of this protein affects the development of the disease or the course thereof.

34. Use of a protein according to one or more of the preceding claims as a growth factor, colony-stimulating factor, a factor inducing erythropoiesis and/or inducing the immune system.

35. Protein according to one or more of the preceding claims,
characterized in that
said protein comprises at least those amino acids which are encoded by nucleotides 74 - 154 or 155 - 685.



08/973,303

SUMMARY

According to the invention, there is disclosed a novel protein having at least differentiation-inducing activity in particular on Friend erythroleukemia cell lines.